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A Conjugation Procedure for *Bdellovibrio bacteriovorus* and Its Use To Identify DNA Sequences That Enhance the Plaque-Forming Ability of a Spontaneous Host-Independent Mutant

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Wild-type bdellovibrios are obligate intraperiplasmic parasites of other gram-negative bacteria. However, spontaneous mutants that can be cultured in the absence of host cells occur at a frequency of 10^{-6} to 10^{-7}. Such host-independent (H-I) mutants generally display diminished intraperiplasmic-growth capabilities and form plaques that are smaller and more turbid than those formed by wild-type strains on lawns of host cells. An analysis of the gene(s) responsible for the H-I phenotype should provide significant insight into the nature of *Bdellovibrio* host dependence. Toward this end, a conjugation procedure to transfer both IncQ and IncP vectors from *Escherichia coli* to *Bdellovibrio bacteriovorus* was developed. It was found that IncQ-type plasmids were capable of autonomous replication in *B. bacteriovorus*, while IncP derivatives were not. However, IncP plasmids could be maintained in *B. bacteriovorus* via homologous recombination through cloned *B. bacteriovorus* DNA sequences. It was also found that genomic libraries of wild-type *B. bacteriovorus* 109J DNA constructed in the IncP cosmid pVK100 were stably maintained in *E. coli*; those constructed in the IncQ cosmid pBM33 were unstable. Finally, we used the conjugation procedure and the *B. bacteriovorus* libraries to identify a 5.6-kb *Bam*HI fragment of wild-type *B. bacteriovorus* DNA that significantly enhanced the plaque-forming ability of an H-I mutant, *B. bacteriovorus* BB5.

Members of the genus *Bdellovibrio* are obligate intraperiplasmic (IP) parasites of other gram-negative bacteria (11, 30, 31). Their unique development cycle consists of two fundamental stages, a free-swimming attack phase and an IP growth phase. While in the attack phase, bdellovibrios are highly motile and metabolically active but they do not replicate their DNA. Upon contact with a host, a bdellovibrio attaches to the outer envelope of the cell and after a short period rapidly penetrates into the host, becoming lodged within the periplasmic space. During the invasion process, the bdellovibrio drops its flagellum and initiates the transition from attack phase to growth phase. Early in the growth phase, the bdellovibrio converts the host cell into a stable spherical structure, termed the bdelloplast, and degrades host cell macromolecules to products that are used for energy generation and cell biosynthesis. DNA replication is initiated about 30 min after invasion. During the remaining period of growth, the bdellovibrio elongates into a coiled, multicellular filament, which, at the cessation of growth, fragments into individual attack-phase cells. The residual bdelloplast is then lysed, and the progeny bdellovibrios are released into the environment.

The transition of a wild-type bdellovibrio from an attack-phase cell to a growth-phase cell is strictly dependent on the availability of a suitable host; all attempts to bypass the host cell requirement with commercial media have been unsuccessful (10, 13, 20, 26). Determining the molecular basis for this host-dependent (H-D) phenotype is critical to an understanding of *Bdellovibrio* growth and development. At present, however, little is known about this aspect of *Bdellovibrio* biology. Several studies have demonstrated that concentrated cellular extracts from hosts and other bacteria can induce wild-type bdellovibrios to enter the growth phase and support the completion of the entire growth cycle (10, 13, 20). Further, Gray and Ruby (11) have hypothesized that at least two different host signals are required to successfully carry out the IP growth cycle: one to trigger differentiation of an attack-phase cell into a growth-phase cell and a second to initiate rounds of DNA synthesis. However, no specific growth factors or signal molecules have been identified, and the mechanism by which the extracts stimulate *Bdellovibrio* growth and multiplication remains unknown.

An important discovery made early in the study of bdellovibrios was that spontaneous mutants that no longer require host cells for growth can be isolated (6, 14, 25, 27, 32). Such host-independent (H-I) mutants complete the transition from attack phase to growth phase and back again on standard complex bacteriological media without any factors that are specifically associated with the IP niche. Upon initial isolation, the majority of these mutants retain limited IP growth capabilities and are termed facultative. When plated on lawns of host cells, facultative H-I mutants form plaques that are smaller and more turbid than plaques formed by wild-type bdellovibrios (6, 25, 32).

H-I mutants have been reported to arise at a frequency of 10^{-6} to 10^{-7} (25, 32), suggesting that single mutational events at one or more loci can obviate the bdellovibrio requirement for host cells. The isolation and characterization of genes affected in H-I mutants should provide significant insight into the nature of *Bdellovibrio* host dependence.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
B. bacteriovorus		
109J	Wild-type	21
109J.1	Sm^r derivative of 109J	This study
109J.2	Rf^r derivative of 109J.1	This study
BB5	H-I derivative of 109J.2	This study
E. coli		
ML35	B *lacI lacY*	22
SR-1	Sm^r Rf^r derivative of ML35	This study
DH5	F^- *endA1 recA1 hsdR17*(r_K^- m_K^+) *deoR thi-1 supE44 gyrA96 relA1*	12
SM10	*supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21 recA* Mu_c^+ RP4-2Tc::Mu; Km^r	28
pKC7	ColE1 Ap^r Km^r	19
pBR328	ColE1 Ap^r Cm^r Tc^r	29
pRK2013	ColE1 Km^r *tra*(RK2)	8
pSUP204	IncQ Ap^r Cm^r Tc^r	18
pSUP304.1	IncQ Ap^r Km^r	18
pMMB33	IncQ Km^r *cos*	9
pRK290	IncP Tc^r	7
pVK100	IncP Tc^r Km^r	15
pVK102	IncP Tc^r Km^r	15
pVKα-1	Derivative of pVK100 containing 445-bp *Hae*II fragment from pUC19	5
pTC3	23.5-kb insert of *B. bacteriovorus* 109J DNA in *Eco*RI site of pVK100	This study
pTC5	4.9-kb insert of *B. bacteriovorus* 109J DNA in *Eco*RI site of pVK100	This study
pTC6	20.5-kb insert of *B. bacteriovorus* 109J DNA in *Eco*RI site of pVK100	This study
pTC7	19.5-kb insert of *B. bacteriovorus* 109J DNA in *Eco*RI site of pVK100	This study
pTC12	5.6-kb *Bam*HI fragment from pTC7 in *Bgl*II site of pVK102	This study
pTC50	0.96-kb *Eco*RI-*Xba*I fragment from pTC12 in *Bam*HI site of pVKα-1	5

This approach, however, has not been exploited because of a lack of systems for genetically manipulating bdellovibrios. Here, we begin to rectify this situation. We identify vectors that can be used to clone *Bdellovibrio bacteriovorus* DNA and describe a conjugation procedure to introduce DNA into both wild-type and H-I strains of *B. bacteriovorus*. We then describe the use of the vectors and conjugation procedure to identify DNA sequences from wild-type *B. bacteriovorus* that enhance the plaque-forming ability of an H-I mutant.

(Portions of this work were presented previously in preliminary form [3, 4].)

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are listed in Table 1. *B. bacteriovorus* 109J and *Escherichia coli* ML35 were obtained from S. C. Rittenberg. All *Bdellovibrio* strains were single-plaque or single-colony purified and stored in 15% glycerol at −80°C.

Media and growth conditions. All *E. coli* cultures were grown at 37°C in Luria-Bertani medium (17). When appropriate, *E. coli* cultures contained the following antibiotics at the indicated concentrations (in micrograms per milliliter): ampicillin, 100; chloramphenicol, 20; kanamycin sulfate, 25; rifampin, 100; streptomycin sulfate, 50; and tetracycline, 12.

IP cultures of *B. bacteriovorus* 109J and its derivatives were grown in dilute nutrient broth (DNB) at 30°C, with *E. coli* ML35 as the host cell. DNB consisted of 1 mM $CaCl_2$, 0.1 mM $MgCl_2$, and 0.8 g of nutrient broth (Difco) per liter. Host cells were prepared by washing overnight cultures of *E. coli* ML35 once in an equal volume of DNB. Liquid IP cultures were established by adding approximately 10^9 host cells and 10^7 bdellovibrios per ml, with single plaques or overnight cultures as the bdellovibrio inoculum. These cultures routinely lysed completely within 24 h. IP cultures were plated for plaque development by adding 0.1 ml of the appropriate bdellovibrio dilution and 10^{10} washed host cells (in 0.3 ml) to 3 ml of an agar overlay (DNB plus 0.7% agar held at 50°C) and immediately spreading the mixture on DNB plates that contained 1.5% agar. Under these conditions, plaques became visible after a 3- to 4-day incubation at 30°C. Rf^r and Sm^r bdellovibrios were usually grown on *E. coli* SR-1 in the presence of 100 μg of rifampin per ml or 50 μg of streptomycin sulfate per ml. Plasmid-containing bdellovibrios were grown on *E. coli* ML35 carrying pKC7 or pBR328, in the presence of 35 μg of kanamycin sulfate per ml or 10 μg of chloramphenicol per ml, respectively.

H-I bdellovibrio cultures were grown at 30°C in PYE medium (25) that contained 10 g of peptone and 3 g of yeast extract per liter. PYE plates were solidified with 1.5% agar. When appropriate, antibiotics were used at the same concentrations as in IP cultures.

Isolation and characterization of an H-I mutant. Spontaneous H-I mutants of *B. bacteriovorus* 109J were obtained at a frequency of 10^{-6} to 10^{-7} by the method of Seidler and Starr (25). Selection for H-I growth yielded yellow CFU that varied in size from barely perceptible to about 3 mm in diameter after a 7-day incubation at 30°C. The spontaneous H-I mutants that formed large colonies could generally be subcultured on solid or liquid PYE medium, whereas those that formed small colonies could not. A well-isolated large-colony mutant, *B. bacteriovorus* BB5, was selected for further characterization. This mutant formed circular, smooth-edged colonies that were about 2 mm in diameter after 7 days of incubation on PYE plates at 30°C. *B. bacteriovorus* BB5 also formed plaques when plated in overlay lawns of host cells on DNB medium, but the plaques were much smaller and more turbid than those formed by wild-type *B. bacteriovorus* 109J (see Results). The total PFU formed by *B. bacteriovorus* BB5 were generally 10 to 100% of the total number of CFU.

Chemicals and reagents. Complex medium components were purchased from Difco. Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs. [α-^{32}P]dCTP (800 Ci/mmol) was purchased from DuPont/New England Nuclear.

Matings. Individual matings were conducted on 3-cm-square pieces of nitrocellulose (Schleicher and Schuell) that were incubated on PYE plates. The nitrocellulose was autoclaved in water, placed on PYE plates, and allowed to dry (30 min at room temperature [RT]). Wild-type *B. bacteriovorus* recipients were prepared from freshly lysed IP cultures. Such cultures were concentrated 10-fold by centrifugation, and 0.1 ml of the suspension was spread on a nitrocellulose filter and allowed to dry (30 min at RT). H-I mutant recipients were prepared by placing 0.1 ml of an overnight culture on a nitrocellulose filter, letting it dry (30

min at RT), and then incubating the filter overnight at 30°C on a PYE plate. Donor *E. coli* cultures that had been washed once in DNB and concentrated 10-fold were spread (0.1 ml) on top of the recipients. After 16 to 24 h of incubation at 30°C, individual matings (nitrocellulose filters) were transferred to 2 ml of DNB and vortexed vigorously and then serially diluted and plated for PFU and CFU. When cultures were plated for axenic growth on PYE plates, streptomycin sulfate (50 μg/ml) was included in the medium to select against growth of the donor. All bdellovibrio recipient cultures were started from −80°C stocks. Donor strains were either *E. coli* SM10 or *E. coli* DH5. For *E. coli* SM10, functions required for the conjugal transfer of IncQ- and IncP-type plasmids were provided by an IncP plasmid that is integrated into the SM10 genome (28). When *E. coli* DH5 was used as the donor, the same transfer functions were provided by the helper plasmid pRK2013 (8), a ColE1 derivative that cannot replicate in *B. bacteriovorus* (2a). When *E. coli* DH5 was the donor, overnight cultures of the strain containing the target plasmid and *E. coli* DH5(pRK2013) were mixed in equal volumes and used as the donor suspension, as described above.

DNA manipulations, Southern analysis, and library construction. Most DNA purification and recombinant DNA methods were standard (23). *B. bacteriovorus* genomic DNA was purified by a CTAB (cetyltrimethylammonium bromide)-based extraction procedure (1).

For Southern analysis, *B. bacteriovorus* genomic DNA was digested with various restriction enzymes, fractionated by electrophoresis in 0.7% agarose gels, and transferred to Nytran membranes (Schleicher and Schuell) by the capillary method. Prior to hybridization, membranes were prewashed in a buffer containing 0.1× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]) and 0.5% sodium dodecyl sulfate (SDS) at 65°C. Prewashed membranes were then prehybridized for 1 h at 68°C in hybridization fluid that contained 6× SSPE, 0.5% SDS, and 0.25% nonfat dry milk (Sanalac). A radiolabeled probe was then added and allowed to hybridize overnight at 68°C. All posthybridization washes contained 0.5% SDS and were done in the following order: twice in 2× SSPE at RT, twice in 0.1× SSPE at RT, and three times in 0.1× SSPE at 68°C. Radiolabeled probes (approximately 10^8 dpm/μg of DNA) were produced by nick translation (kit obtained from Bethesda Research Laboratories) or random priming (23).

B. bacteriovorus 109J genomic libraries were constructed by the method described by Ausubel et al. (1). Genomic DNA was partially digested with *Eco*RI and size fractionated in 0.5% agarose gels, and the DNA fragments ranging in size from 20 to 30 kb were electroeluted from the gel and purified with Elutip-d columns (Schleicher and Schuell). The size-fractionated DNA was ligated into the *Eco*RI site of pVK100 (15), and the products were packaged into lambda particles by using commercial extracts (Promega) according to supplier specifications. Packaged cosmids were transduced into *E. coli* DH5 and stored at −80°C.

RESULTS

Conjugal transfer of RSF1010 and RK2 derivatives into *B. bacteriovorus*. It has been shown previously that RK2 transfer functions supplied in *trans* can effect conjugal transfer of RSF1010 (IncQ)- and RK2 (IncP)-derived plasmids between gram-negative bacteria (7, 18). We therefore attempted to use RK2 transfer functions to conjugally transfer both classes of plasmids from *E. coli* to *B. bacteriovorus* (see Materials and Methods). Matings of *E. coli* carrying RSF1010 derivative pSUP204, pSUP304.1, or pMMB33 with wild-type *B. bacteriovorus* 109J.2 and H-I *B. bacteriovorus* BB5 yielded antibiotic-resistant recipients (Table 2). Kanamycin sulfate (20 to 40 μg/ml) and chloramphenicol (5 to 10 μg/ml) were effective in selecting for transfer of the RSF1010 derivatives, while tetracycline (2 to 25 μg/ml) and ampicillin (5 to 50 μg/ml) were not. Matings conducted in the absence of RK2 transfer functions did not yield antibiotic-resistant bdellovibrios, indicating that plasmid transfer was conjugal in nature. Southern analysis indicated that the RSF1010 derivatives were maintained in the *B. bacteriovorus* strains by autonomous replication: *Bam*HI digestion of total DNA isolated from *B. bacteriovorus* BB5(pMMB33) produced a single 13.8-kb band, the size expected for linear pMMB33, that hybridized with pMMB33 (Fig. 1A). In addition, transformation of *E. coli* DH5 with total DNA from *B. bacteriovorus* BB5(pMMB33) yielded transformants that contained pMMB33 (data not shown).

TABLE 2. Conjugal transfer of plasmids into *B. bacteriovorus*[a]

Plasmid	Transfer frequency[b]	Useful antibiotic(s) for selection
IncQ		
pSUP204	10^{-3}	Chloramphenicol, kanamycin
pSUP304.1	10^{-3}	Kanamycin
pMMB33	10^{-3}	Kanamycin
IncP		
pRK290	ND[c]	
pVK100	ND	
pTC3	10^{-4}	Kanamycin

[a] Data apply to matings involving both H-D and H-I strains as recipients, with both SM10 and DH5(pRK2013) as donors.
[b] Expressed as approximate number of antibiotic-resistant recipients per total number of recipients. Frequencies given are typical of those obtained in numerous experiments.
[c] ND, not detected (less than 10^{-7}).

In contrast to results with the RSF1010-derived plasmids,

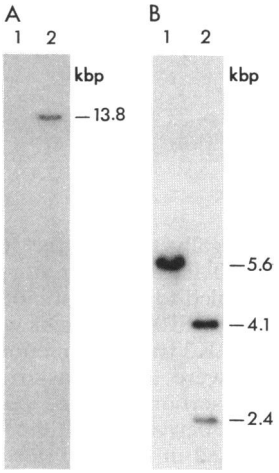

FIG. 1. Southern analysis of plasmid-containing *B. bacteriovorus* strains. (A) *Bam*HI digests of total DNA isolated from *B. bacteriovorus* BB5 (lane 1) and BB5(pMMB33) (lane 2) hybridized with radiolabeled pMMB33. (B) *Bam*HI digests of total DNA isolated from *B. bacteriovorus* BB5 (lane 1) and BB5(pTC50) (lane 2) hybridized with a radiolabeled 5.6-kb *Bam*HI insert from pTC12.

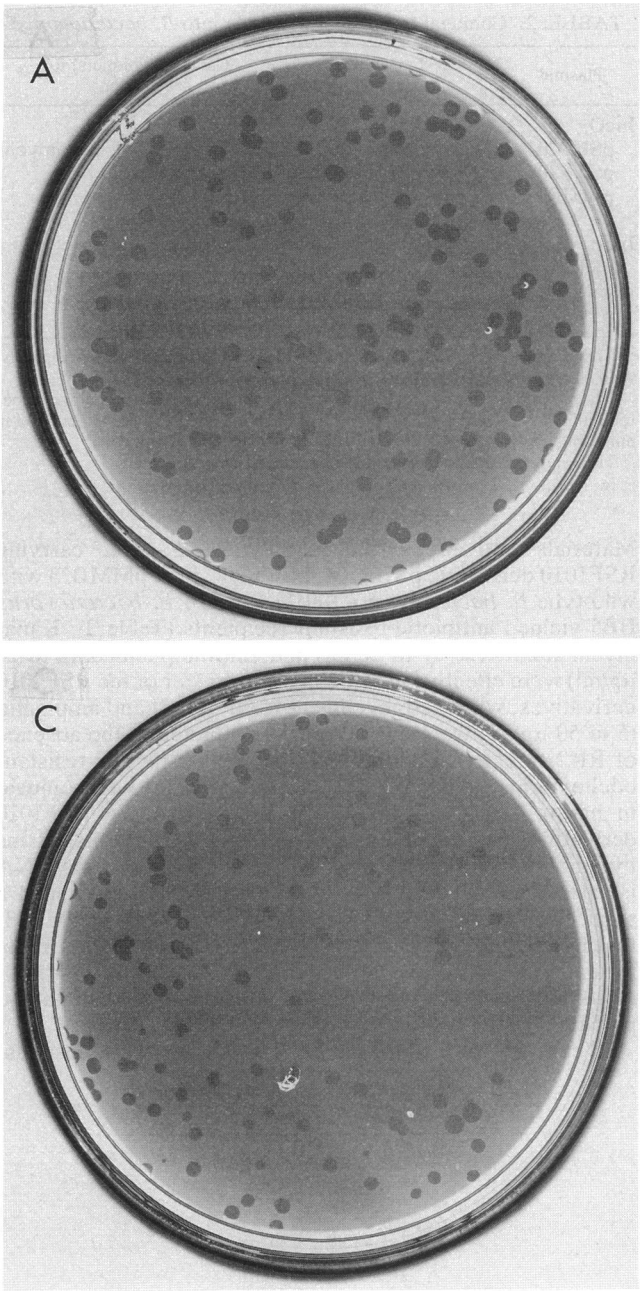

FIG. 2. Plaque phenotypes of *B. bacteriovorus* strains. The plaques formed by *B. bacteriovorus* 109J.2(pTC3) (A), BB5(pTC3) (B), and BB5(pTC12) (C) are shown. pTC3 is used here as a control to confer Km^r; it does not affect the plaque phenotype of wild-type or mutant strains. Overnight IP cultures of each strain yielded about 10^9 PFU/ml (5).

all attempts to conjugally transfer the RK2-based vectors pRK290 and pVK100 into *B. bacteriovorus* 109J and *B. bacteriovorus* BB5 failed to yield antibiotic-resistant recipients (Table 2). Since RSF1010 plasmids were mobilized into *B. bacteriovorus* by RK2 transfer functions, it was possible that RK2 plasmids were also transferred but could not replicate. If true, insertion of a *B. bacteriovorus* DNA sequence into an RK2 derivative could potentially allow the vector to be maintained in bdellovibrios via integration by homologous recombination. Indeed, conjugal transfer of pTC3, a derivative of pVK100 containing a random 23.5-kb fragment of *B. bacteriovorus* DNA (Table 1), produced kanamycin-resistant recipients of both wild-type and H-I *B. bacteriovorus* (Table 2).

Integration of an RK2-based construct into the *B. bacteriovorus* genome was demonstrated by Southern analysis of total DNA obtained from *B. bacteriovorus* BB5(pTC50) (Fig. 1B). pTC50 contains a 0.96-kb *Eco*RI-*Xba*I fragment of *B. bacteriovorus* 109J DNA subcloned from the 5.6-kb *Bam*HI fragment of *B. bacteriovorus* 109J DNA contained in pTC12 (Table 1). The *Xba*I and *Eco*RI termini of the 0.96-kb *Eco*RI-*Xba*I fragment, however, were replaced with *Bam*HI termini prior to cloning into the *Bam*HI site of the RK2-based vector pVKα-1 (5). On the basis of the DNA sequence of the 0.96-kb *Eco*RI-*Xba*I fragment (5), integration of pTC50 into the *B. bacteriovorus* BB5 genome via homologous recombination should have transformed the 5.6-kb *Bam*HI fragment into two *Bam*HI fragments of 4.1 and 2.4 kb. This prediction proved to be the case: total DNA from *B. bacteriovorus* BB5 contained a single hybridizing band representing the 5.6-kb *Bam*HI fragment, while a *Bam*HI digest of *B. bacteriovorus* BB5(pTC50) contained two smaller fragments of 4.1 and 2.4 kb (Fig. 1B).

Identification of wild-type *B. bacteriovorus* DNA sequences that enhance plaque formation by H-I mutant *B. bacteriovorus* BB5. The H-I mutant *B. bacteriovorus* BB5, like previously described facultative H-I mutants (6, 24), retains a limited capacity for IP growth and forms small, turbid plaques on lawns of host cells (Fig. 2B). If this phenotype was due to a mutation that resulted in gene inactivation, then it might be possible to identify the affected gene by transferring a wild-type genomic DNA library into *B. bacteriovorus* BB5 and screening for recombinants with an enhanced plaquing ability (i.e., recombinants that produce large, clear plaques). The wild-type sequences that had been transferred to the enhanced-plaque recombinants could then be isolated and characterized.

To conduct the experiment described above, we first needed to construct a genomic library of *B. bacteriovorus*.

This was initially attempted with the RSF1010-based cosmid pMMB33, but it was found that this vector could not stably maintain large *B. bacteriovorus* DNA inserts (25 to 35 kbp) in *E. coli*. Stable libraries of *B. bacteriovorus* genomic DNA, however, could be constructed with the RK2-based cosmid pVK100. Six independently packaged libraries, VL-1 through VL-6, used in subsequent experiments were composed of about 100 clones each. Given an insert size of between 22 and 28 kbp and a *B. bacteriovorus* genome size of 1×10^6 to 2×10^6 bp (16, 24), it was calculated by the method of Clarke and Carbon (2) that about 360 clones would be required to ensure a 99% probability that any given sequence would be represented in a library. The six libraries were then individually mated into *B. bacteriovorus* BB5, and the recombinants were screened for enhanced plaquing. No enhanced-plaque recombinants were obtained with libraries VL-3 and VL-4 or plasmid pTC3 (which served as the negative control). However, about 1% of the total Km^r recipients obtained with libraries VL-1, VL-2, VL-5, and VL-6 gave rise to plaques that were much larger and clearer than those produced by *B. bacteriovorus* BB5.

If the observed enhancement of *B. bacteriovorus* BB5 plaquing activity resulted from homologous recombination of wild-type sequences into the recipient genome at the site of the H-I mutation, then identical or related cosmids should have been present in the enhanced-plaque recombinants. Southern analysis was conducted to determine whether this was the case. Total DNA from a number of recombinants was digested with *Hin*dIII, an enzyme that cuts once within pVK100, and was subjected to Southern analysis with pVK100 as the probe (Fig. 3). Each digest would be expected to contain two bands that hybridized to the cloning vector, both of which would have extended in opposite directions from the *Hin*dIII site within pVK100 to *Hin*dIII sites in the adjacent bdellovibrio DNA (Fig. 3A). When 18 randomly picked recipients from the VL-1 and VL-2 matings were analyzed, 17 distinct hybridization patterns were observed (Fig. 3B). These results contrasted with those obtained with enhanced-plaque recombinants obtained from the VL-1, VL-2, and VL-5 matings. All of the *B. bacteriovorus* BB5 recombinants that contained cosmids from VL-1 (nine individuals) and VL-2 (eight individuals) displayed the same hybridization pattern, and a second pattern was seen in 10 isolates that contained cosmids from VL-5 (Fig. 3C; the hybridization patterns of six individuals from each mating are presented). Thus, the data indicated that specific regions of the *B. bacteriovorus* 109J genome were involved in conferring the enhanced-plaque phenotype upon *B. bacteriovorus* BB5.

Further analysis of the wild-type DNA sequences responsible for plaque enhancement required their isolation. This was accomplished in several steps. Total DNA from an enhanced-plaque recombinant that showed the predominant restriction fragment hybridization pattern (Fig. 3C, VL-1 lane 5) was digested with *Bam*HI, thereby releasing the vector from the genome with flanking bdellovibrio sequences attached to each end. This linear, vector-containing *Bam*HI fragment was then circularized in a dilute ligation and transformed into *E. coli* DH5. A single plasmid, pTC5, that contained pVK100 plus two flanking *Eco*RI-*Bam*HI fragments of 2.3 and 2.6 kb was identified. The 4.9-kb *Eco*RI insert from pTC5 was then purified and used to probe colony lifts of the VL-1 library. Two cosmids that hybridized to the pTC5 insert, pTC6 and pTC7, were isolated and found to contain overlapping inserts of 20.5 and 19.5 kb, respectively (Fig. 4).

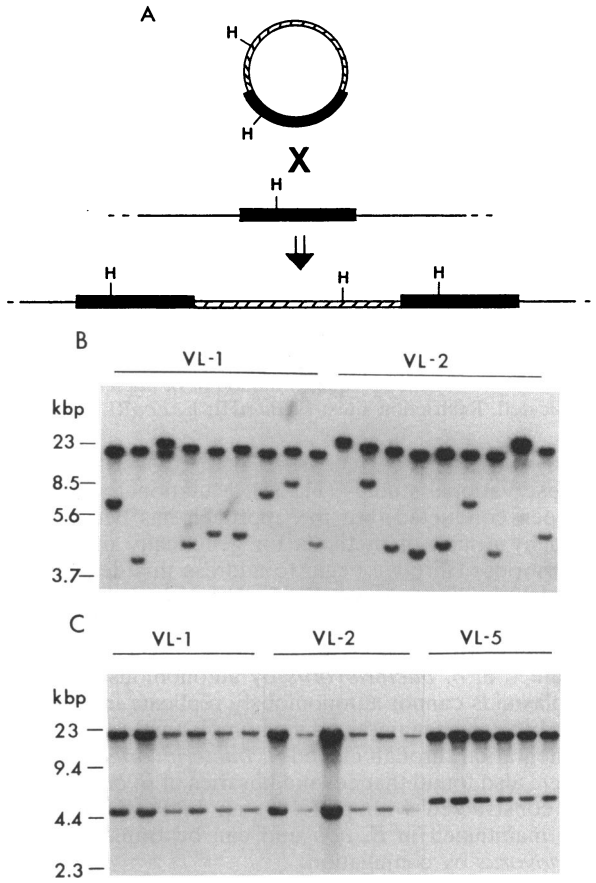

FIG. 3. Southern analysis of cosmid-containing recombinants. (A) Schematic diagram showing cointegration of a cosmid into the recipient *B. bacteriovorus* genome. Thin line, recipient genome; solid box, cloned *B. bacteriovorus* DNA; cross-hatched box, pVK100 vector. Two hypothetical *Hin*dIII fragments that hybridize to the pVK100 probe are shown. H, *Hin*dIII restriction site. (B) Southern analysis of *Hin*dIII digests of total DNA isolated from 18 random *B. bacteriovorus* BB5 recipients hybridized with radiolabeled pVK100. Individual Km^r isolates were obtained after mating with libraries VL-1 and VL-2. (C) Southern analysis of *Hin*dIII digests of total DNA isolated from 18 enhanced-plaque *B. bacteriovorus* BB5 recombinants hybridized with radiolabeled pVK100. Individual Km^r isolates were obtained after mating with libraries VL-1, VL-2, and VL-5.

Recombination of pTC6 into the *B. bacteriovorus* BB5 genome had no effect on the plaquing phenotype of the H-I mutant. However, recombination of pTC7 into *B. bacteriovorus* BB5 did; it conferred the enhanced-plaque phenotype. The region of pTC7 responsible for the phenotype was then further delineated. In particular, the 5.6-kb *Bam*HI fragment of pTC7 was subcloned, yielding pTC12 (Fig. 4), and was found to enhance the plaque-forming ability of *B. bacteriovorus* BB5 (Fig. 2C). The plaques produced by *B. bacteriovorus* BB5 appeared identical to those produced by *B. bacteriovorus* BB5(pTC7) (not shown) and nearly identical to those formed by wild-type *B. bacteriovorus* (Fig. 2A), except possibly for a slight reduction in size.

DISCUSSION

Most of what has been learned about *Bdellovibrio* growth and development has come from biochemical, physiological,

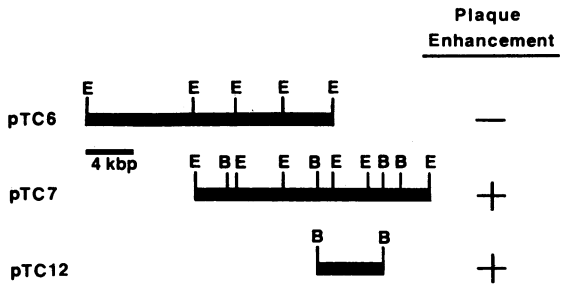

FIG. 4. Abilities of wild-type *B. bacteriovorus* 109J DNA sequences to enhance plaque formation of *B. bacteriovorus* BB5. The DNA inserts in pTC6, pTC7, and pTC12 are shown, and the abilities of the inserts to enhance plaque formation of *B. bacteriovorus* BB5 are indicated. Restriction sites: B, *Bam*HI; E, *Eco*RI.

and observational studies (11, 31). Mutational analysis has also been conducted, but the approach has been severely limited by a lack of methods for genetically manipulating bdellovibrios. Here we begin to address this deficiency. In particular, we have shown that IncQ and IncP plasmids can be conjugally transferred from *E. coli* to both wild-type and H-I mutants of *B. bacteriovorus*. The IncQ plasmids are maintained in *B. bacteriovorus* by autonomous replication. IncP plasmids cannot autonomously replicate in *B. bacteriovorus* but can be maintained via homologous recombination and integration through cloned *B. bacteriovorus* sequences. We have also found that cosmid libraries of *B. bacteriovorus* DNA constructed with pVK100, an IncP vector, can be stably maintained in *E. coli* and can be transferred to *B. bacteriovorus* by conjugation.

We have exploited the ability to transfer DNA from *E. coli* to *B. bacteriovorus* to initiate a genetic analysis of the *Bdellovibrio* requirement for host cells. Specifically, we have identified a 5.6-kb *Bam*HI fragment of wild-type *B. bacteriovorus* 109J DNA that dramatically enhances the plaque-forming capacity of an H-I mutant, *B. bacteriovorus* BB5; whereas *B. bacteriovorus* BB5 forms small, turbid plaques on lawns of *E. coli*, the recombinant *B. bacteriovorus* BB5(pTC12) forms large, clear plaques that closely resemble those formed by wild-type *B. bacteriovorus* 109J (Fig. 2). The basic question raised, of course, is whether *B. bacteriovorus* BB5 contains a mutation within the 5.6-kb *Bam*HI fragment: i.e., was the enhanced-plaque phenotype of *B. bacteriovorus* BB5(pTC12) due to correction of the original mutation or did recombination of the wild-type 5.6-kb *Bam*HI fragment into the genome of *B. bacteriovorus* BB5 indirectly suppress the poor-plaquing phenotype of the mutant? In an accompanying paper (5), we show that the former is the case and define a genetic locus, designated *hit* (host interaction), that has a fundamental role in the *Bdellovibrio*-host cell interaction.

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